Aminated graphene for DNA attachment produced via plasma functionalization

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We demonstrate the use of a unique plasma source to controllably functionalize graphene with nitrogen and primary amines, thereby tuning the chemical, structural, and electrical properties. Critically, even highly aminated graphene remains electronically conductive, making it an ideal transduction material for biosensing. Proof-of-concept testing of aminated graphene as a bio-attachment platform in a biologically active field-effect transistor used for DNA detection is demonstrated. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4711771]

Consisting of only a single layer of carbon atoms, graphene responds dramatically to the presence of adsorbates, making it an attractive material for sensing applications. In its pristine state, graphene is inert, and so for targeted sensing applications the surface must first be functionalized. However, the type, concentration, and distribution of heteroatoms or molecules can greatly alter not only the chemical but also the electronic characteristics. Consider for example, one well-studied chemically modified graphene that has been used for sensing applications: graphene oxide (GO).[1–7] Increasing the oxygen concentration in GO from less than 10 at. % to around 40 at. % converts graphene from hydrophobic to hydrophilic while increasing its resistivity by 10^5 orders of magnitude.[1,2] The increased reactivity is certainly attractive for subsequent chemistries, however, the significant reduction in conductivity can be an impediment in sensor development. Although graphene oxide is cheap and effective in many applications, it contains diverse oxygen-rich functional groups that may or may not participate in a chemical reaction. What is needed for many applications is, chemically modified graphene with a simple and robust functional group that also substantially maintains its conductivity.

In this work, we demonstrate the use of electron beam produced plasmas to functionalize graphene with primary amines (NH_2), a simple yet important functional group for many biosensing and biological applications since they enable numerous covalent reactions.[8] We also demonstrate the chemical flexibility of the aminated graphene by binding it to trifluoroethylamine using glutaraldehyde, and to single stranded DNA (oligonucleotides). Electron beam generated plasmas are ideally suited for the functionalization of graphene in that they possess high densities ($>10^9\,cm^{-3}$) but low electron temperatures (<1 eV).[9] The latter will generate low plasma potentials and thus ions impacting adjacent surfaces will have low kinetic energies.[10] In molecular gases, the ion energies will not exceed ≈3 eV.[10] Such low energies are critical in preserving the carbon basal plane due to the low energy thresholds for vacancy creation, 7.44 eV,[11] and displacement, 35 eV,[12] in graphite. In prior work,[13] these plasmas were shown to preserve graphene's structural integrity after oxygen and fluorine functionalization. Herein, we use electron beam generated plasma produced in an ammonia containing background. Other techniques have employed ammonia as the primary source for the incorporation of nitrogen via annealing[14] or using other plasma sources[15–17] but no information was provided about the primary amine grafting.

The electron beam generated plasma system, described in detail elsewhere,[10] utilizes a sheet-like, 2 keV electron beam to produce large area plasmas, which can uniformly process 300 mm wafers (Figure 1(a)). As the electron beam propagates through the background gas, it creates excited species, radicals, electrons, and ions whose concentrations increase with operating pressure. Beam spreading via collisions with the background gas is minimized by an applied magnetic field (150 G). For these experiments, pulsed plasmas were produced in mixtures of argon (Ar) and ammonia (NH_3), where the NH_3 was 5% by flow. The duty factor was 10% (the ratio of 2 ms pulse width to 20 ms period) and a total treatment time was 60 s (equivalent to a plasma exposure time of 6 s). Graphene was grown by chemical vapor deposition (CVD) on Cu and transferred onto cleaned silicon wafers with 100 nm of thermal oxide, prior to treatment. Those samples were located 2.5 cm from the nominal beam axis.

In Ar/NH_3 plasmas a large number of reactive species are produced by electron impact ionization of NH_3,[18,19] dissociative electron-ion recombination[20] and charge exchange reactions.[21] These species include Ar^+, NH_3^+, NH_2^+ ions along with H_2 and H, N, NH and NH_2 radicals. Given the range of species in the plasma, there are several potential reaction pathways to a primary amine formation–dissociation of NH_3^+ ions on the substrate into NH_2 and H, a reaction between NH_2 ions or radicals and the graphene, or the reaction of H and H_2 with NH and N bound at the surface.

The structural and chemical changes due to the plasma exposure were tracked using Raman (Figure 1(b)) and x-ray photoelectron spectroscopies (Figure 1(c)). The addition of

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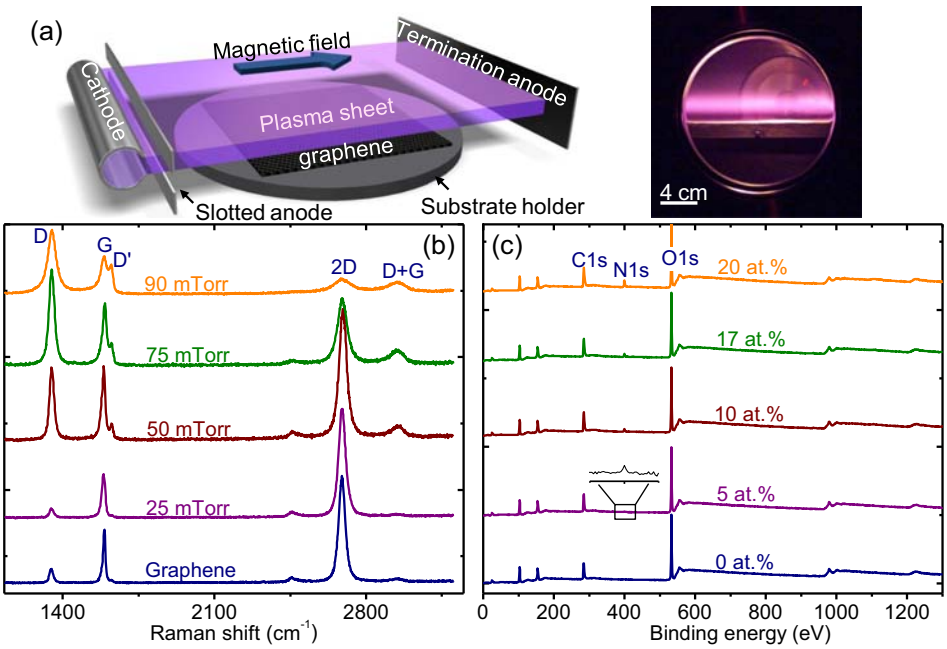

FIG. 1. (a) Three dimensional schematic view (left) and a photograph (right) of the electron beam generated plasma processing system. (b) Raman spectra and (c) its corresponding XPS survey spectra of graphene processed in Ar/NH_3 plasmas at pressures ranging from 25 mTorr to 90 mTorr. The nitrogen concentrations at each pressure are calculated with respect to the total area of the C1s carbon peak.

heteroatoms can be either as substitutions within the graphene plane or as adsorbates on the surface. Raman spectroscopy can resolve the reduction in sp^2 C-C bonds caused by both the increase in sp^3 carbon bonds with surface species or substitution of nitrogen or NH. The G and 2D peaks, associated with the well-ordered sp^2 C-C bonds, decrease with increasing operating pressure. Simultaneously, the D peak (and starting from 50 mTorr, the D′ peak), a signature of disorder, increases with operating pressure (Figure 1(b)). Nitrogen incorporation is the primary driver for these changes, and the XPS survey spectra (Figure 1(c)) show a concurrent increase in nitrogen functionality concentrations from 5 at. % to 20 at. % as the operating pressure increases from 25 mTorr to 90 mTorr (Figure 2(a)). The dose of species (defined here as the total number of species delivered to the surface) increases with pressure and time,[22] and so the increase in surface concentration is expected.

To confirm and quantify the presence of primary amines, chemical tagging using glutaraldehyde that binds trifluoroethylamine to primary amines was employed since the high fluorine content of the covalently attached trifluoroethylamine is readily quantified by XPS. The labeling results are presented as the ratio of fluorine to carbon signal in Figure 2(b) (left axis) and allowed the estimation of the primary amines concentration (right axis in Figure 2(b)). Since the binding efficiency is not known, the primary amine concentration should be considered a lower limit. Nonetheless, the primary amine surface population increases with pressure. For operating pressures of 75 and 90 mTorr, 17% and 45% of the incorporated nitrogen are primary amines, respectively (Figure 2(b)).

Although amination of graphene is a first step toward biofunctionalization, given the predominance of wet chemistry processing, attachment points on graphene would be of little use if they were unstable in water. To test their aqueous stability, the aminated graphene films were washed with deionized water to remove non-covalently bound functional groups. After washing, no change of the C1s and N1s in the high resolution XPS spectra was observed, confirming that the functional groups were stable and covalently bound.

The character of the nitrogen and amine bonding on graphene may be determined to some extent with surface energy measurements. Specifically, using multiple liquids with different polar and dispersive properties can tease out the relative contributions to surface energy. Graphite's hydrophobic character results from the absence of polar components on the surface and so the addition of polar functional groups should reduce this hydrophobicity in proportion to their concentration. The water contact angle of untreated graphene, 98°, is lower than that of graphite, 112°,[23] and can be attributed to the impurities and wrinkles on the graphene surface generated during growth and transfer process steps. After the graphene is plasma treated, the water wetting angle decreased to as low as 52° at 90 mTorr (Figure 2(c)). The use of multiple liquids with different polar and dispersive properties can tease out the relative contributions to surface energy. Those measurements showed a 12 mJ/m^2 increase in the total surface energy due entirely to an increase in the polar component (from 1 mJ/m^2 to 13 mJ/m^2; the dispersive component remained constant at 36 mJ/m^2) from the functional groups attached to the graphene sheet.

In graphene-based device fabrication, one must typically trade-off conductivity against enhanced chemical flexibility. Two point probe measurements (Figure 2(d)) show that the aminated graphene remains highly conductive and thus enables one to avoid such a trade-off. This is surprising due to the tight association between π orbital overlap in sp^2 carbon and conductivity. A change in a carbon atom's hybridization to sp^3 creates scattering sites and can act as a carrier trap that reduces carrier density. Both effects degrade conductivity as seen in previously reported chemically modified graphene such as GO (Refs. 1 and 2) and perfluorographane.[24,25] Nitrogen incorporation differs from oxidation and fluorination in that it can better maintain the conductivity due to electron

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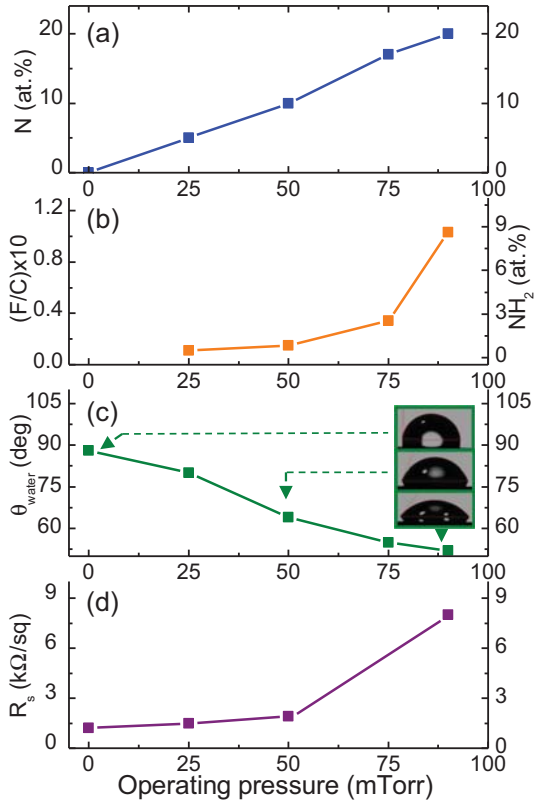

FIG. 2. (a) Nitrogen concentration, (b, left axis) fluorine to carbon ratio, (b, right axis) primary amines concentration, (c) water wetting angle, and (d) sheet resistance of graphene treated in Ar/NH_3 plasmas as a function of operating pressure. The zero of the abscissa represents untreated graphene.

doping. This is borne out in these films. The sheet resistivity increased only by a factor of 6.5 times from 1.2 kΩ/sq to 8 kΩ/sq at the highest concentrations (20 at. %). Fluorinated and oxygenated graphene with comparable covalent functionalization have sheet resistances more than thousands times the starting film.[1,2,24,25] Therefore, while the addition of nitrogen functionalities can lead to the disruption of the π cloud making the graphene surface chemically active (recall the hydrophilic character of the functionalized graphene), the in-plane surface appears to remain conductive. In contrast to functional groups like oxygen or fluorine whose high electronegativity may attract the electron cloud, nitrogen's lower electronegativity appears a better choice to preserve conductivity.

A practical test to confirm the successful amination of CVD-grown graphene by our technique is by implementing standard surface biofunctionalization chemistries to create a functioning biosensor. To test the feasibility of using aminated graphene as a transducing surface, proof-of-concept experiments were performed using a graphene-based, label-free biologically active field-effect transistor (BioFET) device. The layout of the device has been described previously,[24] but in short, a working FET device and a reference FET device are patterned side by side. The working FET is functionalized with single stranded DNA (ssDNA), complementary to the target ssDNA of interest. The reference FET is functionalized with a judiciously randomized, non-complementary strand of ssDNA in order to maintain equal surface charge on both devices. By only functionalizing the

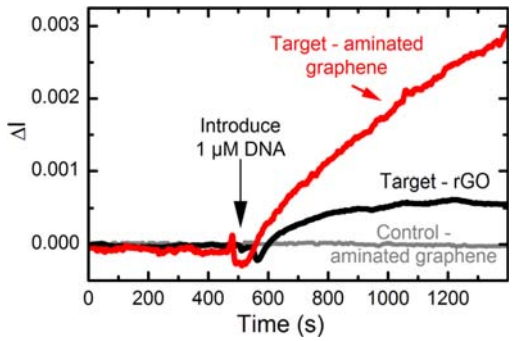

FIG. 3. A comparative graph showing the response of the aminated graphene BioFET device to a solution containing specific, complementary ssDNA molecules (target–aminated graphene) and non-complementary ssDNA molecules (control–aminated graphene). Also shown is response of the previously reported, aminated reduced graphene oxide (target-rGO) device to the same concentration of target ssDNA molecules.

working FET, the signal generated in reference FET represents non-specific interactions and is subtracted out. The probe ssDNA is attached to the surface via an amine group at the 3′ terminus using glutaraldehyde as a bifunctional linker between the amines formed on the graphene surface and the 3′ amine on the ssDNA. During sensing trials, both devices are exposed to the test solution of target ssDNA and a differential measurement is taken representing the relative change in source-drain current between the two FETs. Such a measurement allows the subtraction of noise common to both devices (such as non-specific adsorbents or pH fluctuations) and isolates the change in current caused by specific interaction with the target ssDNA. The transducing surface in previous designs used rGO,[26] which required a robust, yet multiple step process for producing ssDNA functionalized films. In this work, ssDNA was bound to the graphene via the primary amines.

Figure 3 shows the current response to 1 μM solutions of target ssDNA (complementary to the probe ssDNA) and control ssDNA (fully mismatched), as well as a comparison to the response seen for the rGO-based device.[26] Not only does the plasma-produced aminated graphene respond, an improvement in the signal-to-noise ratio compared to the rGO-based device (Figure 3) is observed. The improvement in response could be due to the higher binding efficiencies, lower noise, or both. A forthcoming paper will detail the characteristics of these devices.

In summary, the functionalization of graphene with nitrogen functional groups was carried out using electron beam-generated plasmas produced in Ar/NH_3. The nitrogen concentration was precisely controlled from 5 to 20 at. %. The presence of primary amines was demonstrated by chemical derivative labeling where up to 45% of the incorporated nitrogen was assigned to primary amines. As the functional group concentration was increased, the chemical reactivity of the surface increased while the electrical conductivity decreased. Importantly, the highly aminated graphene remained conductive and as such, amine functionalized graphene was tested as a platform for DNA attachment. Those results demonstrated a higher signal to noise ratio than rGO based devices.

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